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**Citation for published version:**

Ibanez, CE, Schrier, R, Ghazal, P, Wiley, C & Nelson, JA 1991, 'Human cytomegalovirus productively infects primary differentiated macrophages', *Journal of Virology*, vol. 65, no. 12, pp. 6581-8.

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Peer reviewed version

**Published In:**

Journal of Virology

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## Human Cytomegalovirus Productively Infects Primary Differentiated Macrophages†

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Received 28 June 1991/Accepted 29 August 1991

**Monocytes are one of the predominant cell types in the peripheral blood that are infected by human cytomegalovirus (HCMV). Although virus can be detected in these cells in vivo, HCMV replication in cultured monocytes has been unsuccessful. In this study, we demonstrate efficient HCMV replication in cultured monocytes. HCMV permissiveness in these cells was dependent on nonadherent cell-induced stimulation of the monocyte, with subsequent morphological differentiation into macrophages. Approximately 40% of the cells infected by virus were detected by immunofluorescent staining with both immediate-early and late antibodies. In addition, viral plaque assays demonstrated significant productive infection of macrophages. These observations are consistent with the suggestion that the monocyte/macrophage serves as a source of viral amplification and dissemination.**

Human cytomegalovirus (HCMV) infection of immunocompromised patients often results in acute disease (10). The virus may also contribute to the immune dysfunction observed in these individuals by directly infecting lymphoid cells (3, 19). HCMV immunosuppression is exemplified by a depressed lymphocyte response to T-cell mitogens (3, 13, 27, 32), decreased natural killer cells (11, 28), and cytotoxic T-lymphocyte activities in *in vitro* studies (27). The T cell may also be functionally impaired without being productively infected (2, 12, 28). This observation is attributed to suppression of various monocyte accessory cell functions including suppression of interleukin 1 production (12, 14, 23) and depressed T-cell proliferative responses to mitogens, such as concanavalin A (ConA) (3, 19, 21) or pokeweed (19, 21), because of HCMV infection. Therefore, cells of the peripheral blood may serve as a target as well as a potential source of viral persistence.

With the advances in immunocytochemical techniques and *in situ* hybridization, cell types naturally infected by HCMV have been identified in the peripheral blood (20) and tissues (8, 15) of acutely infected individuals. Studies of separated cell populations from the peripheral blood in natural or *in vitro* HCMV infections have demonstrated monocytes (5, 12, 19), T cells (1, 6), and neutrophils (27, 32) as the predominant infected cell types. In HCMV asymptomatic patients, viral infection of peripheral blood cells is infrequent and expression appears to be restricted to early events (1, 4, 5, 19). However, in patients with acute HCMV infection, virus can be frequently isolated from these cells (3, 20, 24). Polymorphonuclear cells may harbor viral DNA; however, expression has not been detected, suggesting that these cells may phagocytize viral particles (32). Examination of biopsies from transplanted organs of patients with HCMV disease indicates that mononuclear inflammatory cells are the predominant infected cell type (8, 36). Both the frequency and the extent of viral expression in infected infil-

trating cells are increased in these tissues relative to the incidence of virus in cells of the peripheral blood. Since infiltrating cells are in a state of activation, the increased incidence of virus detected in these cells may be due to cellular induction of viral replication.

Cellular differentiation has been observed to correlate with productive HCMV infection in a few cell lines. For example, the monocytic cell line THP-1 is permissive for HCMV after 12-*O*-tetradecanoylphorbol-13-acetate-induced differentiation of the monocyte into a macrophage (34). Similar results were also observed with the teratocarcinoma cell line (N-Tera-2) (9). In these systems, HCMV replicates in differentiated but not undifferentiated cells. These observations might suggest that either differentiation events are linked to HCMV activation or the virus productively infects only terminally differentiated cells.

HCMV infection of peripheral blood mononuclear cells *in vitro* has demonstrated viral expression in a few cells restricted to early viral events. One of the major cell types infected by HCMV in these cultures is monocytes (1, 4, 5, 19). A variety of different culture methods have been developed by several groups to study the growth of HCMV and other viruses in primary monocyte/macrophage cultures (2, 3, 7, 11, 18, 19, 30). Most of these culture systems are heavily reliant on continuous exposure of various cytokines and mitogens in the growth medium, but to date none of these primary culture systems have been successful in replicating HCMV. Recently, a primary monocyte/macrophage culture system which relies on antigenically or mitogenically activated T-cell contact with monocytes was described (25). Stimulated monocytes differentiate into multinucleated giant cells (MNGCs) and can be maintained in culture for months without the addition of exogenous cytokines. This differentiation pathway may mimic the activation of monocytes into macrophages observed *in vivo* by antigen-specific reaction with activated T cells. We have employed these monocyte/macrophage culture techniques on cells derived from normal donors to examine HCMV replication in the differentiating cells. We demonstrate that HCMV can productively infect monocyte/macrophages, depending on the stage of morphological differentiation.

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† Publication no. 6883-IMM from the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA.

## MATERIALS AND METHODS

**Isolation and culture of macrophages.** Peripheral blood mononuclear cells were isolated from the blood of HCMV seronegative donors screened by an enzyme-linked immunosorbent assay. All blood donors were arranged through the General Clinic Research Center at the Scripps Clinic and Research Foundation. The cells were collected by underlaying heparinized treated whole blood with Histopaque (Sigma Chemical Co., St. Louis, Mo.) and centrifuged at 1,500 rpm in a Beckman T6 centrifuge for 25 min at room temperature. The peripheral blood mononuclear cell band was collected, washed twice with sterile saline and once with serum-free media, and resuspended to 4 million cells per ml in Iscove's medium (GIBCO Laboratories, Grand Island, N.Y.) with penicillin-streptomycin and 10% pooled human serum from laboratory personnel seronegative for human immunodeficiency virus (HIV) and HCMV. Approximately 5 million nonadherent and adherent cells were plated onto Primaria culture dishes (60 by 15 mm) (Becton Dickinson, Lincoln Park, N.J.) and incubated at 37°C with 7.5% CO<sub>2</sub>. Unstimulated adherent cells were allowed to adhere for 90 min, at which time all nonadherent cells were removed with incomplete 60/30 medium (60% AIM V, 30% Iscove's medium [both from GIBCO], penicillin-streptomycin) and replaced with 3 ml of fresh complete 60/30 medium containing 10% pooled human serum. Adherent cells stimulated for differentiation were treated with the addition of ConA (10 µg/ml) and incubated for 20 h. The following day, all nonadherent cells were removed and 3 ml of fresh complete 60/30 medium was added. Every 4 or 5 days, the adherent monocyte cultures were fed complete 60/30 medium by replacing one-half of the old medium with fresh medium. Day 1 of differentiation is defined as the day after the initial peripheral blood mononuclear cell isolation and stimulation with ConA.

**Characterization of adherent monocyte cultures.** The adherent cells were greater than 98% esterase positive at 72 h. The nonspecific esterase staining was performed by using a kit from Sigma. Cells were also characterized as monocytic by immunocytochemistry with the monoclonal antibodies Leu M1, M3, 3a, and 3b and found to be 98 to 100% positive for these markers (Becton Dickinson and Co., Mountain View, Calif.). In these studies, approximately 70% of the monocyte cultures differentiated into MNGCs. The remaining monocyte cultures either did not differentiate or self-differentiated without ConA stimulation. This observation was associated with either the batch of human serum used or specific cell donor variation. For the purposes of this study, only cultures that were 98% esterase positive and exhibited appropriate differentiation with ConA and lymphocyte stimulation were used.

**In vitro infection of monocyte/macrophages.** The recent isolate of HCMV, I-G, (19) was used to infect primary cultures of monocyte/macrophages. The frozen stock of I-G, originally isolated from an adult patient with mononucleosis, had been passaged through human fibroblasts (HFF) and frozen at passage 12 in liquid nitrogen. Consequently, frozen samples from this stock were thawed and passaged for one to five additional rounds through HFF cells prior to monocyte/macrophage infections. Sonicated HCMV-infected HFF cell lysates were used as the source of virus, which was diluted 1:1 with monocyte/macrophage-conditioned medium. Monocyte/macrophage cultures containing  $5 \times 10^5$  cells per dish (60 by 15 mm) were infected at various days during differentiation with infected lysates containing approximately  $10^8$

PFU per dish. The virus was allowed to adhere for 12 h, washed, and then replaced with conditioned medium.

**Immunofluorescence of CMV immediate early and late antigen.** In vitro HCMV-infected monocyte/macrophages were fixed in methanol and reacted at a dilution of 1:100 with immediate-early (IE) (L-14) (19) and late (L) (J-24) monoclonal antibodies for 2 h at 37°C. The J-24 monoclonal antibody was characterized by its ability to detect antigen late in the HCMV life cycle. The kinetic appearance of the antigen occurred after viral DNA synthesis (unpublished results). The secondary antibody (Becton Dickinson)—goat anti-mouse fluorescein conjugate (1:100) was reacted with the HCMV monoclonal antibodies for 1 h at 37°C. The infected monocyte/macrophages used for immunofluorescence were cultured in four chamber plastic slides (Nunc Inc., Naperville, Ill.).

**Viral absorption and penetration assays.** Five millicuries of [*methyl*-1,2-<sup>3</sup>H]thymidine (100 to 130 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) was used to metabolically label virions in six 162-cm<sup>2</sup> flasks of HFF cells after an initial 36 h of infection. The cells were incubated with [<sup>3</sup>H]thymidine for 48 h with fresh medium containing 2% dialyzed fetal calf serum. The infected cells were then sonicated, and the cellular debris was pelleted. Labeled virus in the supernatant was pelleted through a 20% cushion of sorbitol Tris-buffered saline (pH 8.0) for 90 min in a Sorvall SA 600 rotor at 16,000 rpm and then further purified through a six-step sorbitol gradient ranging from 20 to 70%. Gradients were spun in a Beckman SW41 rotor for 2 h at 18,000 rpm. The white opulent viral band was collected between the 50 and 60% interface and diluted 10-fold with 60/30 macrophage medium. Approximately equal numbers of macrophage cells ( $2.2 \times 10^4$ ) and HFF cells ( $5.6 \times 10^4$ ) were infected with 100,000 cpm of <sup>3</sup>H-labeled HCMV. An initial 4-h incubation period in minimal volume was performed to allow for adherence; then the infected cells were harvested, and additional medium was collected 12 h after the initial infection. The infected cultures were then fractionated into four fractions, and the levels of radioactivity were assessed in a Beckman LS 5000TD scintillation counter. The first fraction (free virus) consisted of medium pooled with one saline wash of the cells. The second fraction (attached virus) consisted of virus released from the surface by incubation with trypsin for 5 min. The cells were again washed thoroughly, and the trypsin-released virus and wash were combined. The third fraction (intercellular virus) consisted of supernatant from the Dounce-homogenized cells pelleted through a sucrose gradient. The fourth fraction (nuclear viral DNA) consisted of the nuclei pellet resuspended in saline. The radioactivity was counted with a hemacytometer. The number of nuclei isolated was used to normalize the radioactive counts in each of the fractions tested. HFF cells were infected with labeled virus to assess virulence. Labeled virus was found to be infectious by the development of a cellular cytopathic effect.

**Viral titer assay.** HFF and unstimulated and stimulated macrophage cultures were infected as previously described. After 20 h, infected cells were washed and fresh 60/30 medium was added. On various days postinfection, medium and cells were then collected in aliquots and frozen at -70°C. At the end of a collection period, the cells and medium were then thawed, sonicated, and plated at various dilutions between 10<sup>-1</sup> and 10<sup>-7</sup> onto six-well plates containing HFF cells at approximately 80% confluency. After an initial 1 h of adherence, ME-medium 199 (GIBCO) solution containing 0.5% SeaKem agarose, 4% glutamine, 10% fetal calf serum, and penicillin-streptomycin was overlaid, and

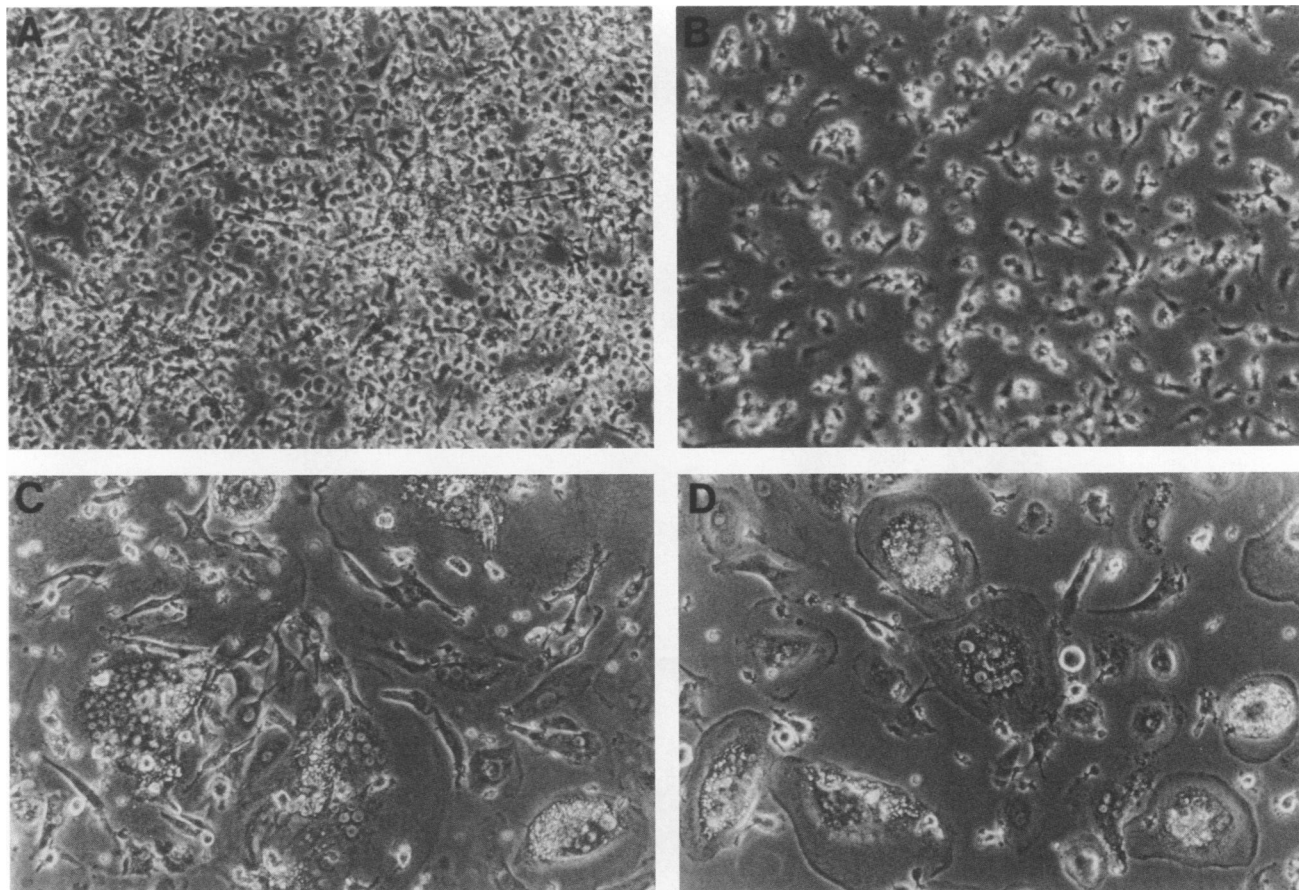


FIG. 1. Morphological differentiation of monocytes into MNGCs. Peripheral blood monocytes were isolated from an HCMV-seronegative donor and stimulated by contact with activated nonadherent cells, as described in Materials and Methods. Cells were cultured for 1 (A), 4 (B), 9 (C), and 14 (D) days poststimulation with the nonadherent fraction. MNGCs are seen in the 9- and 14-day cultures. The formation of MNGCs may occur between 6 and 15 days poststimulation depending on the donor. Magnification,  $\times 100$ .

the cultures were allowed to incubate for 10 to 12 days, with occasional feeding. Cells were fixed with a 25% formalin-phosphate-buffered saline solution for 10 to 15 min and stained with a 0.05% solution of methylene blue (Ricca Chemicals) (35).

## RESULTS

**Macrophage culture characteristics.** The morphological differentiation of the monocyte cultures was based on a 20-h stimulation of the monocyte-derived adherent cells and the lymphocyte-derived nonadherent cell fractions in the presence of 10  $\mu\text{g/ml}$  of ConA. A fivefold increase or decrease in the concentration of ConA resulted in detachment of adherent cells within several days of initial stimulation. After the initial stimulation and washing of adherent cells, a 30 to 40% fraction of adherent cells, referred to as short-term adherents (29, 37), detached from the surface of the plate and were removed by further washes (Fig. 1A). This process occurred over a period of 3 to 4 days poststimulation (Fig. 1B). The remaining attached cells slowly increased in size (Fig. 1C), and some fused and formed MNGCs (Fig. 1D). Cells at the two later stages were greater than 98% esterase positive

(Fig. 2) and could be maintained in culture for several months. MNGCs were observed as early as 6 or as late as 25 days poststimulation. Individual donors demonstrated different rates and frequencies of MNGC formation. The cellular morphology associated with activated nonadherent cell-induced differentiation of monocytes was similar to that of long-term primary macrophage cultures previously reported (29, 37).

**Expression of HCMV in human primary macrophage cultures infected in vitro.** The ability of HCMV to infect primary macrophage cultures stimulated by contact with activated nonadherent cells was examined at various times poststimulation. For these studies, the low-passage, recent isolate of HCMV, I-G, was used to infect monocyte/macrophage cultures at days 1, 4, and 16 poststimulation. Cells were fixed at various days postinfection and tested for the presence of the HCMV IE and L antigens by immunofluorescence. The results presented are derived from four experiments with monocyte cultures from four individual donors exhibiting various rates of differentiation of the monocyte cultures. The precise kinetics for the appearance of viral antigens varied from donor to donor, but a correlation was observed between increased frequencies of cells expressing viral antigens and the overall degree of morphological differentiation



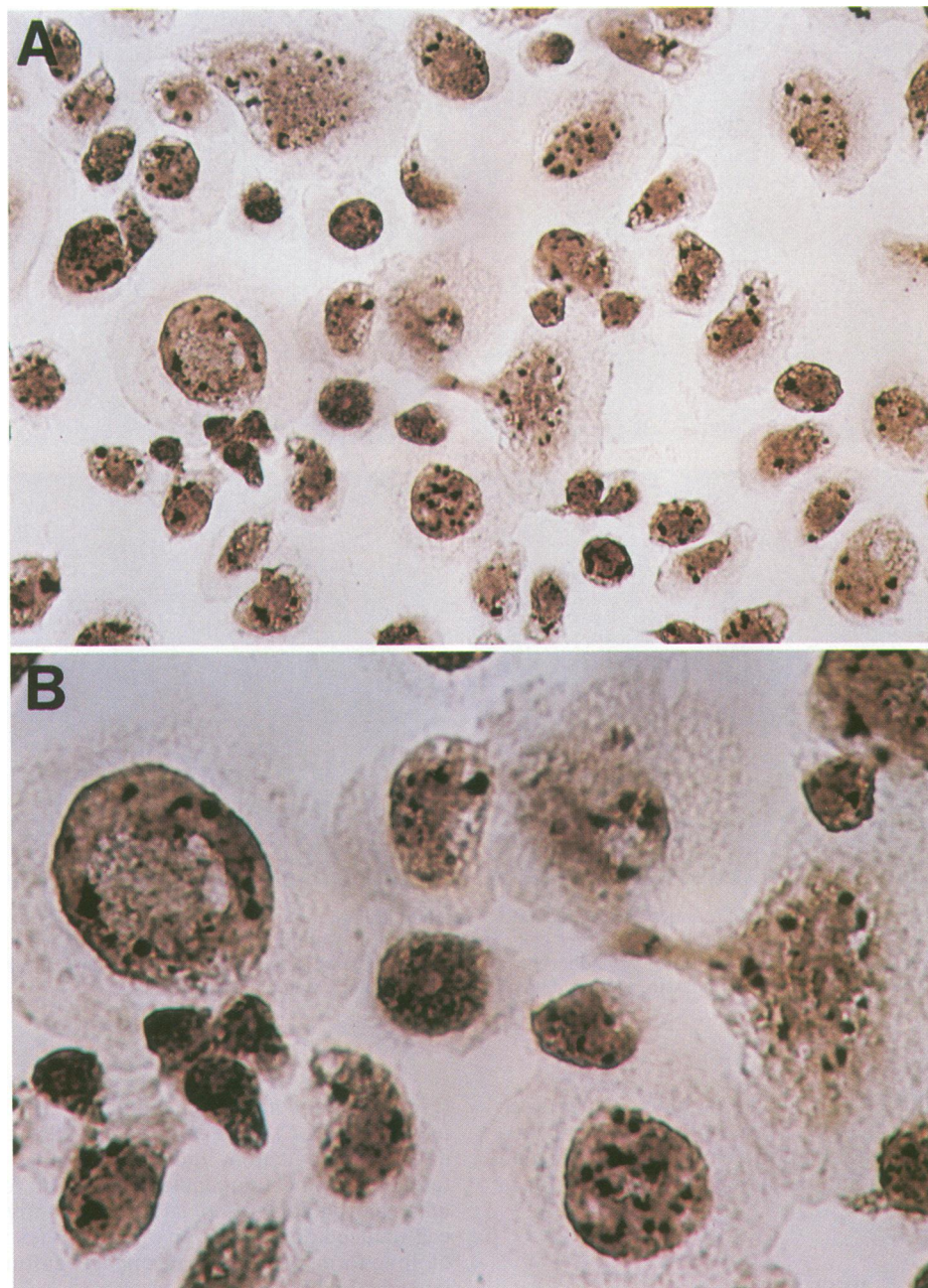


FIG. 2. The presence of esterase in macrophage cultures. Peripheral blood monocytes stimulated by contact with activated nonadherent cells were stained after 6 days for the presence of nonspecific esterase activity. The charcoal granules characteristic of esterase activity was observed in 98% of the cells. Cells were counterstained with hematoxylin. Magnifications,  $\times 100$  (A) and  $\times 200$  (B).

of the monocyte/macrophage culture. Day-1-infected cultures tested for the presence of IE antigen demonstrated little or undetectable levels of the protein at 1, 6, and 13 days postinfection (data not shown). We observed that infection of early differentiating cells (1 to 3 days poststimulation), prior to the formation of MNGCs, often impaired or inhibited further morphologic differentiation. When monocytes were infected at day 4 poststimulation, approximately 1% of the cells at 24 h postinfection demonstrated the presence of IE antigen by immunofluorescence. The percentage of cells with IE antigen increased to 20% as morphologic differenti-

ation occurred at 6 to 13 days postinfection (Fig. 3B, C, and E). L antigen was detected in day-4-infected cultures and paralleled the increase in the percentage of IE-positive cells (Fig. 3D). The 16-day-old macrophages in which MNGCs composed 2 to 4% of the cultures also demonstrated higher frequencies of infectivity without any noticeable cytopathic effect. These cultures demonstrated the greatest degree of morphological differentiation and exhibited infectivity rates of up to 40% of the cells at 6 to 13 days postinfection by immunofluorescence for the presence of IE and L antigens. A quantitative representation of the above data is given in



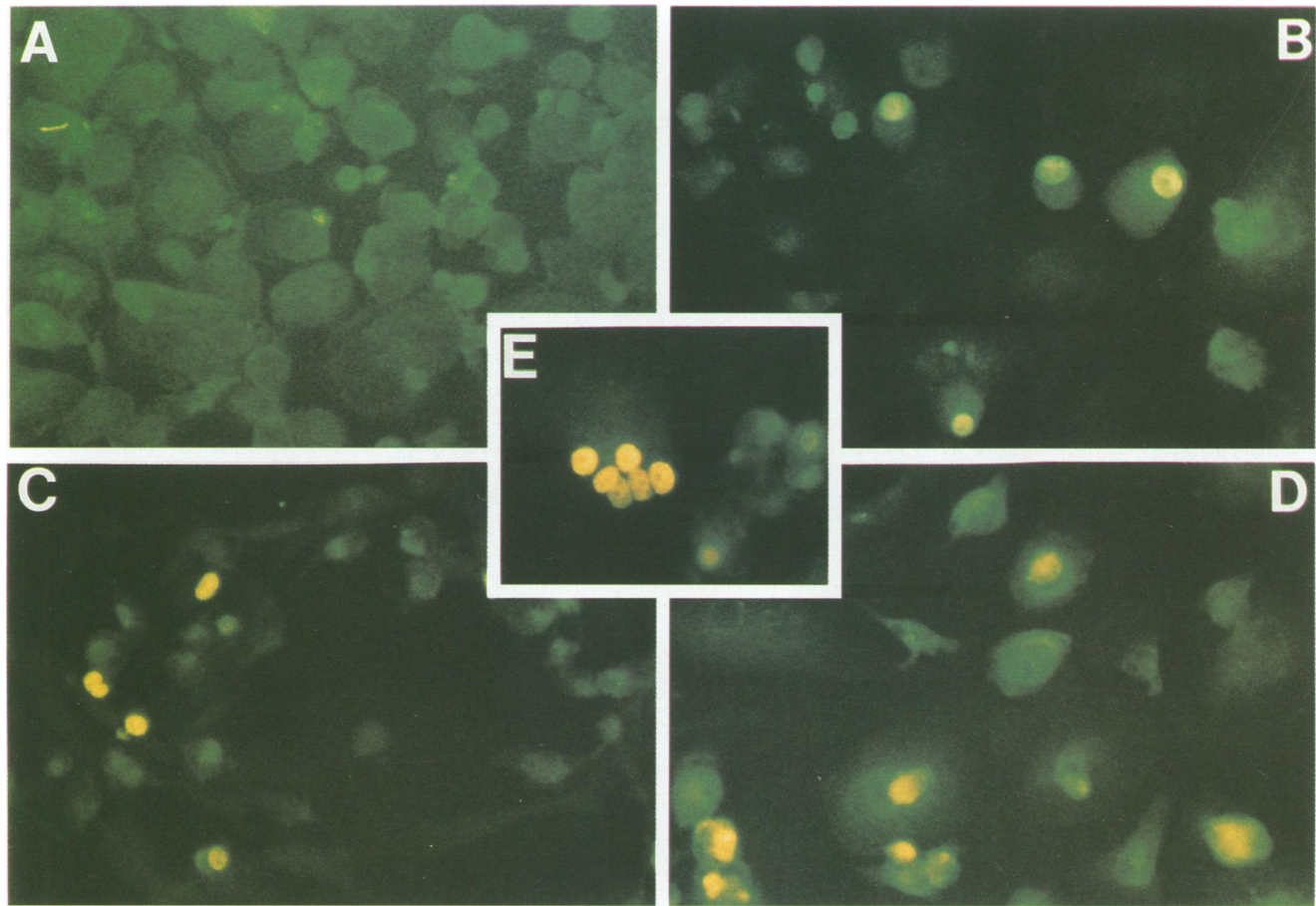


FIG. 3. The presence of HCMV proteins in macrophage cultures. HCMV-infected macrophages were stained by immunofluorescence for the presence of IE (B, C, and E) and L (D) viral antigens by using mouse monoclonal antibodies and techniques as described in Materials and Methods. The characteristic IE staining was observed in the nucleus of macrophages (B and C) and MNGCs (E). L viral antigen was also observed with these cells (D). Mock-infected cultures did not stain for IE (A) or L (data not shown) antigens. Magnification,  $\times 200$ .

Table 1, which demonstrates the frequency of HCMV infection of macrophage cultures infected and harvested at various days postinfection for one individual donor.

**Virus penetration in 1-day cultures.** To determine whether the lack of HCMV antigen expression in day-1-infected monocyte cultures was due to the inability of the virus to attach to and penetrate the cells, monocytes were infected with HCMV containing  $^3\text{H}$ -labeled genomic DNA. Day 1 monocytes and permissive HFF cells of equivalent cell numbers were infected with equal counts of labeled virus ( $1 \times 10^5$  cpm). At 12 h postinfection, cells were washed,

fractionated, and assayed for the presence of  $^3\text{H}$ -labeled viral DNA in the nucleus and cytoplasm and on the cell surface. Upon harvest and quantitation, 75 to 80% of the label was recovered from both cultures. Approximately 3% of the label was found in the cytoplasmic fraction or attached to the surface of the monocytes, while 6% of the labeled virus was recovered from these fractions of HFF cells. After the counts found in each fraction were normalized to the number of nuclei isolated, similar amounts of  $^3\text{H}$ -labeled viral DNA were recovered from the nuclei of both the permissive HFF cells and the nonpermissive day 1 monocyte/macrophages (Table 2). Therefore, the results suggest that viral absorption and penetration are not involved in the lack of HCMV expression observed in day 1 monocyte/macrophage cultures.

**HCMV productively infects differentiated macrophages.** The presence of late HCMV gene products in the infected morphologically differentiated macrophage cultures suggested that the virus productively infected these cells. To test this hypothesis, a series of one-step viral growth analysis was performed with infected cell lysates and supernatants from unstimulated monocytes as well as day 1 and day 10 nonadherent-cell-activated monocyte/macrophages. Cells infected with HCMV demonstrated little cytopathic effect. Viral plaque assays of HFF with day-10-infected monocyte/

TABLE 1. Frequency of HCMV infection in macrophage cultures

| Day of differentiation <sup>a</sup> | % of cells positive for viral antigen at postinfection day |              |                 |
|-------------------------------------|--|--------------|-----------------|
|                                     | 1  | 6            | 13              |
| 1                                   | 0  | 0            | 0               |
| 4                                   | <1   | $\approx 20$ | NT <sup>b</sup> |
| 16                                  | <1   | <1           | $\approx 40$    |

<sup>a</sup> Macrophage cultures were infected at either day 1, 4, or 16 poststimulation with activated nonadherent cells.

<sup>b</sup> NT, not tested.

TABLE 2. Ability of HCMV to attach and penetrate monocytes

| Culture             | Virus (cpm/10 <sup>4</sup> nuclei) <sup>a</sup> |               |         |
|---------------------|---|---------------|---------|
|                     | Attached  | Intracellular | Nuclear |
| HFF                 | 1,100   | 490           | 80      |
| Monocyte/macrophage | 1,400   | 800           | 77      |

<sup>a</sup> Monocyte cultures were infected 1 day poststimulation with activated nonadherent cells with <sup>3</sup>H-labeled HCMV. At 12 h postinfection, cells were washed, fractionated, and assayed for the presence of <sup>3</sup>H-labeled viral DNA in the nucleus and cytoplasm and on the cell surface with a scintillation counter.

macrophage cell lysates demonstrated the highest levels of viral replication titers so far reported, with titers ranging from 10<sup>5</sup> to 10<sup>7</sup> PFU/ml (Fig. 4). Each time point on the graph represents approximately 4 × 10<sup>5</sup> cells per infected dish and at the peak of infection averages a titer of 25 PFU per cell. The highest titers of virus in the macrophage cultures again correlated with the maturity of the stimulated monocyte/macrophage cultures, as evidenced by the formation of MNGCs. In contrast, HCMV infection of day-1-stimulated cultures resulted in a 4-to-5-log decrease in viral titers. The infection of unstimulated cultures produced minimal amounts of virus (Fig. 4). To determine whether virus was released from infected cells, supernatants were assayed for the presence of HCMV. Virus was not detected in any of the above-described supernatants, suggesting that HCMV may be predominantly cell associated in the monocyte/macrophages (data not shown).

## DISCUSSION

**Replication of HCMV in monocyte/macrophages.** This study demonstrates that HCMV can productively infect primary monocyte/macrophages and is the first report of the

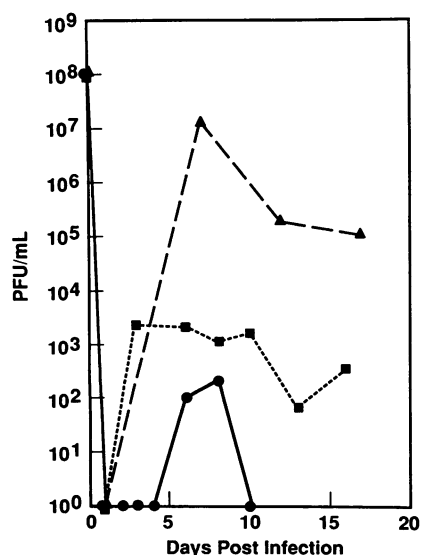


FIG. 4. An HCMV one-step growth curve for infected macrophages. Macrophage cultures were infected at a multiplicity of infection of 100 at days 0 (●), 1 (■), and 10 (▲) poststimulation with activated nonadherent cells, as described in Materials and Methods. Cells were harvested at the indicated times, and viral titers were determined by plaque assay of HFF.

productive replication of HCMV in primary cells from the hematopoietic system. This report supplies direct evidence that the monocyte/macrophage may play an important role in the biology of HCMV and addresses the requirement of cellular activation and differentiation as a prerequisite for HCMV expression and permissiveness in this cell type.

Mature differentiated cultures of monocyte/macrophages exhibiting the presence of MNGCs generated the highest titers (10<sup>7</sup> PFU/ml) of HCMV without the development of any discernible cytopathic effect. However, monocyte/macrophage cultures infected at either day 0 or day 1 produced an amount of HCMV that was 4 to 5 logs lower than that of day-10-infected cultures. In agreement with these observations, the immunofluorescence data demonstrated a high percentage of cells expressing viral antigens at the later stages of differentiation. Although minimal viral replication was detected in the viral titer assays, the immunofluorescence experiments showed no detectable viral expression in day-1- and day-0-infected cultures. A possible explanation for the apparently conflicting observations may be related to the levels of sensitivity inherent in the different assays. Alternatively, the low levels of viral replication in the early monocyte/macrophages may indicate subpopulations of monocytes that have spontaneously differentiated. Spontaneously differentiating monocytes may have arisen by prior stimulation *in vivo* or at some point during the culturing procedure. In our experience, this differentiated subpopulation makes up approximately 0.01% of the total culture.

Supernatants from HCMV productively infected macrophages did not contain virus, suggesting that HCMV may be tightly cell associated in these cultures. Similar results with HIV in which the virus is restricted to monocyte vesicles that fail to egress from the cell have been reported (18). This phenomenon may be related to either HIV strain differences or a unique property of the macrophage. Future studies with other HCMV strains will determine whether strict cell association is a common feature of virus infection of macrophages.

Previous studies of HCMV infection of primary monocyte/macrophage cultures have reported low frequencies of infection, with expression restricted to early viral events (1, 4, 5, 19). The inability to replicate HCMV in these primary human mononuclear cell cultures has been hypothesized to be due to the limited survival time of these cells *in vitro* (22). Most macrophage culture systems rely on the addition of various cytokines (granulocyte-macrophage or macrophage colony-stimulating factor or tumor necrosis factor), which may produce varying degrees of stimulation. In the system described in this report, activated nonadherent cell stimulation of the monocyte for 20 h is sufficient to induce morphological differentiation and produce cultures that survive up to several months without further stimulation. Our ability to maintain these differentiated cells in culture for extended periods of time may explain our success in infecting these cells.

Prior studies that used this culture system have suggested that the T lymphocyte is, most likely, the cell in the nonadherent cell population responsible for stimulation of the monocyte. This conclusion is based on experiments in which antibodies directed against the DR locus of class II restriction blocks monocyte differentiation as well as HIV reactivation (25). This T-cell-monocyte requirement is believed to mimic the T-cell-monocyte interactions that occur *in vivo* and, therefore, provides an excellent model system for studying viral monocyte/macrophage interactions.

**Differentiation and HCMV replication.** The importance of differentiation as a prerequisite for HCMV replication has

been demonstrated in other cell systems. For example, when the monocyte cell line THP-1 is treated with 12-*O*-tetradecanoylphorbol-13-acetate, the cells exhibit a macrophage phenotype (31). Undifferentiated THP-1 cells are resistant to HCMV infection, whereas differentiated cells are permissive for virus replication (34). The human teratocarcinoma cell line N-Tera-2 is another example of how differentiation dramatically affects the fate of HCMV replication. In this cell line, HCMV replicates in differentiated but not undifferentiated cells (9). Similar to that of primary macrophages, the timing of HCMV infection of N-Tera-2 cells during the differentiation process is crucial for production of virus. When cells are infected early in the differentiation process, a limited amount of virus is detectable. Maximal amounts of HCMV are produced only in terminally differentiated cells. This observation parallels the monocyte/macrophage system in which optimal amounts of virus are recovered from older macrophage cultures. The block in HCMV replication in the N-Tera-2 cell line occurs at the transcriptional level of the major IE promoter, which regulates the transcriptional activator genes of the virus (17). A similar mechanism may also regulate HCMV expression in the monocyte/macrophages. Studies with labeled virus show that the block in HCMV expression in nonpermissive unstimulated monocytes is not at the level of absorption and penetration into the cell. Other posttranscriptional events, however, may regulate viral replication in these cells.

**The role of the monocyte/macrophage in HCMV biology.** The observation that HCMV can productively infect monocyte/macrophages suggests that the virus can directly perturb an important component of the immune system as well as serve as a vector for viral dissemination into various tissues. However, we have been unable to reactivate HCMV from the monocyte/macrophage cultures of HCMV-seropositive patients, suggesting that monocytes are not latent sources of virus (unpublished observations). Our results would indicate that permissiveness of macrophages to HCMV infection is dependent on the extent of cellular differentiation. Therefore, macrophages may serve as a source of viral amplification. Thus, similar to those of HIV, T cells and monocytes play a critical role in the biology of HCMV. The viruses, however, may utilize the cells for different purposes. Whereas the monocyte is a reservoir and vector for HIV, it appears to serve as a vector and amplification unit for HCMV. Future studies will be required to understand the mechanisms involved in T-cell-induced expression of HCMV in the monocyte.

#### ACKNOWLEDGMENTS

This work was supported in part by USPHS grants AI-21640 and AI-24178 (J.A.N.), MO1 RR00833 (GCRC), and NIH NINCDS NS-25178 and NS-27810 and funds provided by the University of California and allocated on the recommendation of the University-wide Task Force on AIDS (C.A.W., R.D.S., P.G.). J.A.N. is the recipient of a faculty award from the American Cancer Society.

We thank Richard Landes and Emilia Glezer for technical assistance and Margaret Stone for manuscript preparation.

#### REFERENCES

- Braun, R. W., and H. C. Reiser. 1986. Replication of human cytomegalovirus in human peripheral blood T cells. *J. Virol.* **60**:29-36.
- Buchmeier, N. A., and N. R. Cooper. 1989. Suppression of monocyte functions by human cytomegalovirus. *Immunology* **66**:278-283.
- Carney, W. P., and M. S. Hirsch. 1981. Mechanisms of immunosuppression in cytomegalovirus mononucleosis. *J. Infect. Dis.* **144**:47-54.
- Dudding, L. R., and H. M. Garnett. 1987. Interaction of strain AD169 and a clinical isolate of cytomegalovirus with peripheral monocytes: the effect of lipopolysaccharide stimulation. *J. Infect. Dis.* **155**:891-896.
- Einhorn, L., and A. Ost. 1984. Cytomegalovirus infection of human blood cells. *J. Infect. Dis.* **149**:207-214.
- Garnett, H. M. 1982. Isolation of human cytomegalovirus from peripheral T cells of renal transplant patients. *J. Lab. Clin. Med.* **99**:92-97.
- Gendelman, H. E., J. M. Orenstein, M. A. Martin, C. Ferrua, R. Mitra, T. Phipps, L. A. Wahl, H. C. Lane, A. S. Fauci, D. S. Burke, D. Skillman, and M. S. Meltzer. 1988. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. *J. Exp. Med.* **167**:1428-1441.
- Gnann, J. W., Jr., J. Ahlmen, C. Svalander, L. Olding, M. B. A. Oldstone, and J. A. Nelson. 1988. Inflammatory cells in transplanted kidneys are infected by human cytomegalovirus. *Am. J. Pathol.* **132**:239-248.
- Gonczol, E., P. W. Andrews, and S. A. Plotkin. 1984. Cytomegalovirus replicates in differentiated but not undifferentiated human embryonal carcinoma cells. *Science* **224**:159-161.
- Griffiths, P. D., and J. E. Grundy. 1988. The status of CMV as a human pathogen. *Epidemiol. Infect.* **100**:1-15.
- Kapasi, K., and G. P. A. Rice. 1986. Role of the monocyte in cytomegalovirus-mediated immunosuppression *in vitro*. *J. Infect. Dis.* **154**:881-884.
- Kapasi, K., and G. P. A. Rice. 1988. Cytomegalovirus infection of peripheral blood mononuclear cells: effects on interleukin-1 and -2 production and responsiveness. *J. Virol.* **62**:3603-3607.
- Levin, M. J., C. R. Rinaldo, P. L. Leavy, J. A. Zaia, and M. S. Hirsch. 1979. Immune response to herpes virus antigens in adults with acute cytomegalovirus mononucleosis. *J. Infect. Dis.* **140**:851-857.
- Moses, A. V., and H. M. Garnett. 1990. The effect of human cytomegalovirus on the production and biologic action of interleukin-1. *J. Infect. Dis.* **162**:381-388.
- Myerson, D., R. C. Hackman, J. A. Nelson, D. C. Ward, and J. K. McDougall. 1984. Widespread presence of histologically occult cytomegalovirus. *Hum. Pathol.* **15**:430-439.
- Nelson, J. A., J. W. Gnann, and P. Ghazal. 1990. Regulation and tissue specific expression of human cytomegalovirus. *Curr. Top. Microbiol. Immunol.* **154**:77-103.
- Nelson, J. A., and M. Groudine. 1986. Transcriptional regulation of the human cytomegalovirus major immediate-early gene is associated with induction of DNase I-hypersensitive sites. *Mol. Cell. Biol.* **6**:452-461.
- Orenstein, J. M., M. S. Meltzer, T. Phipps, and H. E. Gendelman. 1988. Cytoplasmic assembly and accumulation of human immunodeficiency virus types 1 and 2 in recombinant human colony-stimulating factor-1-treated human monocytes: an ultrastructural study. *J. Virol.* **62**:2578-2586.
- Rice, G. P. A., R. D. Schrier, and M. B. A. Oldstone. 1984. Cytomegalovirus infects human lymphocytes and monocytes: virus expression is restricted to immediate-early gene products. *Proc. Natl. Acad. Sci. USA* **81**:6134-6138.
- Rinaldo, C. R., Jr., P. H. Black, and M. S. Hirsch. 1977. Interaction of cytomegalovirus with leukocytes from patients with mononucleosis due to cytomegalovirus. *J. Infect. Dis.* **136**:667-678.
- Rinaldo, C. R., Jr., W. P. Carney, B. S. Richter, P. H. Black, and M. S. Hirsch. 1980. Mechanisms of immunosuppression in cytomegalovirus mononucleosis. *J. Infect. Dis.* **141**:488-495.
- Rinaldo, C. R., Jr., B. S. Richter, P. H. Black, R. Callery, L. Chess, and M. S. Hirsch. 1978. Replication of herpes simplex virus and cytomegalovirus in human leukocytes. *J. Immunol.* **120**:130-136.
- Rodgers, B. C., D. M. Scott, J. Mundin, and J. G. P. Sissons. 1985. Monocyte-derived inhibitor of interleukin 1 induced by human cytomegalovirus. *J. Virol.* **55**:527-532.
- Saltzman, R. L., M. R. Quirk, and M. C. Jordan. 1988. Disseminated cytomegalovirus infection. *J. Clin. Invest.* **81**:75-81.



25. Schrier, R. D., J. A. McCutchan, J. C. Venable, J. A. Nelson, and C. A. Wiley. 1990. T-cell-induced expression of human immunodeficiency virus in macrophages. *J. Virol.* **64**:3280–3288.
26. Schrier, R. D., J. A. Nelson, and M. B. A. Oldstone. 1985. Detection of human cytomegalovirus in peripheral blood lymphocytes in a natural infection. *Science* **230**:1048–1051.
27. Schrier, R. D., and M. B. A. Oldstone. 1986. Recent clinical isolates of cytomegalovirus suppress human cytomegalovirus-specific human leukocyte antigen-restricted cytotoxic T-lymphocyte activity. *J. Virol.* **59**:127–131.
28. Schrier, R. D., G. Rice, and M. B. A. Oldstone. 1986. Suppression of NK activity on T cell proliferation induced by fresh isolates of cytomegalovirus. *J. Infect. Dis.* **153**:1084–1091.
29. Sechler, J. M., M. K. Warren, and J. I. Gallin. 1989. Human non-transformed monocyte-derived macrophage cell lines. *J. Immunol. Methods* **119**:277–285.
30. Sung, S., and J. A. Walters. 1989. Identification and characterization of a hamster monoclonal antibody anti-2.28 directed against a 70-kilodalton activation antigen in human monocytes. *J. Immunol.* **142**:1903–1908.
31. Tsuching, S., Y. Kobayashi, Y. Goto, H. Okumura, S. Nahae, and K. Tada. 1982. Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. *Cancer Res.* **42**:1530–1537.
32. Turtinen, L. W., R. Saltzman, M. C. Jordan, and A. T. Haase. 1987. Interactions of human cytomegalovirus with leukocytes *in vivo*: analysis by *in situ* hybridization. *Microb. Pathog.* **3**:287–297.
33. Wahren, B., P. Ljungman, T. Paulin, and O. Ringden. 1986. Enhancive and suppressive effects of cytomegalovirus on human lymphocyte responses *in vitro*. *J. Virol.* **58**:909–913.
34. Weinshenker, B. G., S. Wilton, and G. P. A. Rice. 1988. Phorbol ester-induced differentiation permits productive human cytomegalovirus infection in a monocytic cell line. *J. Immunol.* **140**:1625–1631.
35. Wentworth, B. B., and L. French. 1970. Plaque assay of cytomegalovirus strains of human origin (35031). *Proc. Soc. Exp. Biol. Med.* **135**:253–258.
36. Wiley, C. A., R. D. Schrier, F. J. Denaro, J. A. Nelson, P. W. Lampert, and M. B. A. Oldstone. 1986. Localization of cytomegalovirus proteins and genome during fulminant central nervous system infection in an AIDS patient. *J. Neuropathol. Exp. Neurol.* **4**:127–139.
37. Zuckerman, H., S. K. Ackerman, and S. D. Douglas. 1979. Long-term human peripheral blood monocyte cultures: establishment, metabolism and morphology of primary human monocyte-macrophage cell cultures. *J. Immunol.* **38**:401–411.